

WHAT IS CLAIMED IS:

1. A method for site-specific incorporation of derivatized
5 dideoxynucleotides into DNA comprising reacting an archaeon Family B
DNA polymerase, a primed DNA template and nucleotide solution containing
at least one derivatized dideoxynucleotide to produce fragments of DNA
with the derivatized dideoxynucleoside covalently attached to the 3' terminal
10 residue wherein the derivatized dideoxynucleotide is incorporated more
efficiently than the corresponding underivatized dideoxynucleotide.

2. A method for site-specific incorporation of acyclonucleotides
into DNA comprising reacting an archaeon Family B DNA polymerase, a
15 primed DNA template and nucleotide solution containing at least one
acyclonucleotide to produce fragments of DNA with the acyclonucleotide
covalently attached to the 3' terminal residue.

3. A method for site-specific incorporation of derivatized
20 acyclonucleotides into DNA comprising reacting an archaeon Family B DNA
polymerase, a primed DNA template and nucleotide solution containing at
least one derivatized acyclonucleotide to produce fragments of DNA with the
derivatized acyclonucleotide covalently attached to the 3' terminal residue.

4. The method of claims 1 or 3 where the derivative comprises a
25 detection reagent.

5. The method of claims 1 or 3 where the derivative comprises a
dye-label.

6. The method of claims 1 or 3 where the derivative comprises a
30 dye selected from the group consisting of TAMRA, ROX, R6G, Fluorescein-
12, IRD40, IRD700, BODIPPY®TR, BODIPY®TMR, BODIPY®R6G and
BODIPY®FI.

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7. The method of claims 2 or 3 where the acyclonucleotide is radioactively labeled.

5 8. The method of claims 1-3 wherein the DNA polymerase has at least about 20% primary amino acid sequence identity with Vent® DNA polymerase.

10 9. The method of claims 1-3 wherein the DNA polymerase has at least about 30% primary amino acid sequence identity with Vent® DNA polymerase.

15 10. The method of claims 1-3 wherein the DNA polymerase has at least about 70% primary amino acid sequence identity with Vent® DNA polymerase.

11. The method of claims 1-3 wherein the DNA polymerase binds to an antibody probe that has antigenic specificity to Vent® DNA polymerase.

20 12. The method of claims 1-3 wherein the DNA polymerase is encoded by an isolated DNA fragment that hybridizes in a Southern blot to an isolated DNA fragment selected from the group consisting of a DNA fragment having nucleotides 1-1274 of SEQ ID NO:4, a DNA fragment having nucleotides 291-1772 of SEQ ID NO:4, a DNA fragment having
25 nucleotides 3387-3533 of SEQ ID NO:4, a DNA fragment having nucleotides 4704-5396 of SEQ ID NO:4, and a DNA fragment having nucleotides 4718-5437 of SEQ ID NO:4, wherein hybridization is conducted under the following conditions: a) hybridization: 0.75 M NaCl, 0.15 M Tris, 10 mM EDTA, 0.1% sodium pyrophosphate, 0.1% sodium lauryl sulfate,
30 0.03% BSA, 0.03% Ficoll 400, 0.03% PVP and 100 µg/ml boiled calf thymus DNA at 50°C for about 12 hours and; b) wash: 3X30 minutes with 0.1X SET, 0.1% SDS, 0.1% sodium pyrophosphate and 0.1 M phosphate buffer at 45°C.

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13. The method of claims 1-3 wherein the DNA polymerase is selected from the group consisting of Vent®, Deep Vent®, *Pfu* and 9°N™ DNA polymerase.

5 14. The method of claims 1-3 wherein the DNA polymerase has been mutated by substitution of the conserved amino acid residue corresponding to Vent® DNA polymerase A488, L492, A493 or Y499.

10 15. The method of claims 1-3 wherein the DNA polymerase has been mutated by substitution of the amino acid residue corresponding to Vent® DNA polymerase A488 by L, I, V, F, S or C.

15 16. The method of claims 1-3 wherein the DNA polymerase has been mutated by substituting the amino acid residue corresponding to Vent® DNA polymerase A488 by L.

20 17. The method of claims 1-3 wherein the DNA polymerase has been mutated by substitution of the amino acid residue corresponding to Vent® DNA polymerase Y499 to L.

25 18. The method of claims 1-3 wherein the DNA polymerase is selected from the group consisting of Vent® (A488L), Vent® (Y499L) and 9°N™ (A485L) DNA polymerases.

30 19. The method of claims 2 or 3 wherein the extent of acyclonucleotide incorporation is greater than that of the corresponding dideoxynucleotide.

35 20. The method of claims 2 or 3 wherein the extent of acyclonucleotide incorporation is at least, approximately, two-fold greater than incorporation of the corresponding dideoxynucleotide.

21. The method of claims 2 or 3 wherein the extent of acyclonucleotide incorporation is at least, approximately, five-fold greater than incorporation of the corresponding dideoxynucleotide.

22. The method of claims 2 or 3 wherein the extent of acyclonucleotide incorporation is at least, approximately, nine-fold greater than incorporation of the corresponding dideoxynucleotide.

23. The method of claims 2 or 3 wherein the extent of ROX-acyclo-CTP incorporation is greater than that of ROX-ddCTP.

24. The method of claims 2 or 3 wherein the extent of ROX-acyclo-CTP incorporation is at least, approximately, two-fold greater than that of ROX-ddCTP.

25. The method of claims 2 or 3 wherein the extent of ROX-acyclo-CTP incorporation is at least, approximately, five-fold greater than that of ROX-ddCTP.

26. The method of claims 2 or 3 wherein the extent of ROX-acyclo-CTP incorporation is at least, approximately, ten-fold greater than that of ROX-ddCTP.

27. The method of claims 1-3 wherein the DNA polymerase is additionally thermostable.

28. The method of claims 1-3 wherein the DNA polymerase has no detectable exonuclease activity.

29. The method of claims 1-3 wherein the DNA polymerase has less than about 5% of the exonuclease activity of the unmodified enzyme.

30. The method of claims 1-3 wherein the DNA polymerase has less than about 25% of the exonuclease activity of the unmodified enzyme.

31. The method of claims 1-3 further comprising the step of employing the resulting sequence-specific termination product or products in DNA sequence determination.